

U.S. Application No. 09/853,581  
Amendment dated February 28, 2005  
In reply to the final office action of December 14, 2004  
Attorney Ref. No.: 037003 - 0280617

## I. AMENDMENT

### Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 44, 48, 52, 53, 57, and 62-64 are amended, and claim 47 is canceled.

1-43. (Canceled)

44. (Currently amended) A method of treating cancer comprising administering to a patient in need thereof:

(a) an admixture comprising a cancer or tumor antigen expressed by cells of the cancer to be treated and a microfluidized antigen formulation comprising:

- (i) a stabilizing detergent,
- (ii) a micelle-forming agent, and
- (iii) a biodegradable and biocompatible oil,

said antigen formulation being formulated as a stable oil-in-water emulsion;

wherein said admixture is administered to said patient in an amount sufficient to induce a cytotoxic T-lymphocyte response in said patient which is specific for the cancer or tumor antigen contained in said admixture, and

(b) a therapeutically effective amount of at least one agent which is capable of neutralizing, blocking, antagonizing, or down regulating the activity or preventing activation of transforming growth factor  $\beta$  (TGF $\beta$ ) specifically, which agent is selected from the group consisting of an anti-TGF $\beta$  antibody, a TGF $\beta$ R-fusion protein, a TGF $\beta$  analog, a TGF $\beta$  binding protein, and a TGF $\beta$ R blocking antibody;

wherein the antigen-containing admixture and the at least one agent which is capable of neutralizing, blocking, antagonizing, or down regulating the activity or preventing activation of TGF $\beta$  are administered sequentially or concurrently, and in any order.

U.S. Application No. 09/853,581  
Amendment dated February 14, 2005  
In reply to the final office action of December 14, 2004  
Attorney Ref. No.: 037003 - 0280617

45. (Previously presented) The method of claim 44, wherein the antigen-containing admixture and the at least one agent which is capable of neutralizing, blocking, antagonizing, or down regulating the activity or preventing activation of TGF $\beta$  are administered sequentially.

46. (Previously presented) The method of claim 44, wherein the antigen-containing admixture is administered intradermally, intramuscularly or subcutaneously and the at least one agent which is capable of neutralizing, blocking, antagonizing, or down regulating the activity or preventing activation of TGF $\beta$  is administered intravenously.

47. (Canceled)

48. (Currently amended) The method of claim ~~47~~ 44, wherein the at least one agent which is capable of neutralizing, blocking, antagonizing, or down regulating the activity or preventing activation of TGF $\beta$  is a thrombospondin peptide or a TGF $\beta$ R Fc-fusion protein.

49. (Previously presented) The method of claim 44, wherein the admixture comprises a cancer or tumor antigen selected from the group consisting of gp100, MART-1/Melan A, gp75, tyrosinase, melanoma proteoglycan, MAGE, BAGE, GAGE, RAGE, N-acetylglucosaminyltransferase-V, mutated B-catenin, mutated MUM-1, mutated cyclin dependent kinases-4, p21 ras, BCR-abl, p53, p185 HER2/neu, mutated epidermal growth factor receptor, carcinoembryonic antigens, carcinoma associated mutated mucins, Epstein Barr nuclear antigen (EBNA) gene products, papillomavirus E7 protein, papillomavirus E6 protein, prostate specific antigens, prostate specific membrane antigen, and prostate carcinoma tumor antigen-1 (PCTA-1).

50. (Previously presented) The method of claim 44, wherein the cancer is selected from the group consisting of breast cancer, brain cancer, cervical cancer, leukemia, lymphoma, prostate cancer, skin cancer, colon cancer, lung cancer, ovarian cancer, pancreatic

U.S. Application No. 09/853,581  
Amendment dated February 14, 2005  
In reply to the final office action of December 14, 2004  
Attorney Ref. No.: 037003 - 0280617

cancer, liver cancer, bladder cancer, kidney cancer, myeloma, colorectal cancer, nasopharyngeal carcinoma, or endometrial cancer.

51. (Previously presented) The method of claim 44, wherein the detergent is provided in an amount ranging from approximately 0.05 to 0.5%.

52. (Currently amended) The method of claim ~~52~~ 51, wherein the amount of detergent is about 0.2%.

53. (Currently amended) The method of claim 44, wherein the detergent is selected from the group consisting of ~~TWEEN-80~~ polyoxyethylene sorbitan monooleate, ~~TWEEN-20~~ polyoxyethylene sorbitan monolaurate, ~~TWEEN-40~~ polyoxyethylene sorbitan monopalmitate, ~~TWEEN-60~~ polyoxyethylene sorbitan monostearate, ~~Zwittergent-3-12~~ n-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, ~~TEEPOL-HB7~~ secondary sodium alkyl sulfate and ~~SPAN-85~~ sorbitane trioleate.

54. (Previously presented) The method of claim 44, wherein the micelle-forming agent has a hydrophile-lipophile balance of between 0 and 2.

55. (Previously presented) The method of claim 44, wherein the amount of the micelle-forming agent ranges from 0.5 to 10%.

56. (Previously presented) The method of claim 55, wherein the amount of the micelle-forming agent ranges from 1.25 to 5%.

57. (Currently amended) The method of claim 44, wherein the micelle-forming agent is selected from the group consisting of: ~~pelexamer 401~~, ~~PLURONIC L62L6~~, ~~PLURONIC L101~~, ~~PLURONIC L64~~, ~~PEG1000~~, ~~TETRONIC 1501~~, ~~TETRONIC 150R1~~, ~~TETRONIC 701~~, ~~TETRONIC 901~~, ~~TETRONIC 1301~~ and ~~TETRONIC 130R1~~.

U.S. Application No. 09/853,581  
Amendment dated February 14, 2005  
In reply to the final office action of December 14, 2004  
Attorney Ref. No.: 037003 - 0280617

(a) a block copolymer consisting of two ethylene oxide (EO) block polymers, each joined to a different end of a propylene oxide (PO) block polymer selected from:

- (i) a PO block polymer having molecular weight of about 3956 and constituting about 90 % of the total molecular weight of said block copolymer;
- (ii) a PO block polymer having molecular weight of about 2000 and constituting about 80 % of the total molecular weight;
- (iii) a PO block polymer having molecular weight of about 3416 and constituting about 90 % of the total molecular weight; and
- (iv) a PO block polymer having molecular weight of about 1740 and constituting about 60 % of the total molecular weight;

(b) polyethylene glycol of average molecular weight of about 1000;

(c) a tetrafunctional block copolymer consisting of ethylenediamine, the two nitrogens of which are each attached to two PO block polymers, each of which is in turn attached to an EO block polymer, wherein said PO block polymers are selected from:

- (i) PO block polymers having combined molecular weight of about 7000 which constitute about 90 % of the total molecular weight;
- (ii) PO block polymers having combined molecular weight of about 2200 which constitute about 90 % of the total molecular weight;
- (iii) PO block polymers having combined molecular weight of about 3300 which constitute about 90 % of the total molecular weight; and
- (iv) PO block polymers having combined molecular weight of about 5500 which constitute about 90 % of the total molecular weight;

(d) a tetrafunctional block copolymer consisting of ethylenediamine, the two nitrogens of which are each attached to two EO block polymers, each of which is in turn attached to a PO block polymer, wherein said PO block polymers are selected from:

- (i) PO block polymers having combined molecular weight of about 6700 which constitute about 90 % of the total molecular weight; and
- (ii) PO block polymers having combined molecular weight of about 5700 which constitute about 90 % of the total molecular weight.

U.S. Application No. 09/853,581  
Amendment dated February 14, 2005  
In reply to the final office action of December 14, 2004  
Attorney Ref. No.: 037003 - 0280617

58. (Previously presented) The method of claim 44, wherein the amount of oil ranges from 1 to 10%.

59. (Previously presented) The method of claim 58, wherein the amount of oil ranges from 2.5 to 5%.

60. (Previously presented) The method of claim 44, wherein the oil exhibits a melting temperature of less than 65°C.

61. (Previously presented) The method of claim 44, wherein the oil is selected from the group consisting of squalane, eicosane, tetratetracontane, pristane, and vegetable oils.

62. (Currently amended) The method of claim 44, wherein the antigen-containing admixture comprises ~~TWEEN-80~~ polyoxyethylene sorbitan monooleate, poloxamer 401 a block copolymer consisting of two EO block polymers, each joined to a different end of a PO block polymer having molecular weight of about 3956 and constituting about 90% of the total molecular weight of said block copolymer, and squalane.

63. (Currently amended) The method of claim 44, wherein the antigen-containing admixture contains no more than 20 micrograms of an immunostimulating ~~peptide~~ muramyl dipeptide.

64. (Currently amended) The method of claim 44, wherein the antigen-containing admixture lacks an immunostimulating ~~peptide~~ muramyl dipeptide.